JC02 Rec'd PCT/PTO 28 MAR 2002

FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

# TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 5686

U.S. APPLICATION NO.: 10/089231

| INTERNATIONAL APPLICATION NO. | INTERNATIONAL FILING DATE | PRIORITY DATE CLAIMED |
|---|---|---|
| PCT/DE00/02753 | August 12, 2000 | September 28, 1999 |

TITLE OF INVENTION: DEVICE AND METHOD FOR CHARACTERIZING SPHEROIDS

APPLICANT(S) FOR DO/EO/US: Hagen THIELECKE and Andrea ROBITZKI

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)) (Unexecuted)
10. ☒ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

**Items 11 to 20 below concern document(s) or information included:**

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items of information:

    International Preliminary Examination Report With Amended Claims 1 and 7-10.
    Application Data Sheet.

| U.S. APPLICATION NO. 10/089231 | INTERNATIONAL APPLICATION NO. PCT/DE00/02753 | ATTORNEY'S DOCKET NO. 5686 |
|---|---|---|

21. ☒ The following fees are submitted:

CALCULATIONS FOR PTO USE ONLY

JC13 Rec'd PCT/PTO 28 MAR 2002

**BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):**
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO.....$1,040

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO.....$ 890

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....$ 740

International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4).....$ 710

International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4).....$ 100

**ENTER APPROPRIATE BASIC FEE AMOUNT =** $ 890.00

| | | | | | |
|---|---|---|---|---|---|
| Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)). | | | | $ 130.00 | |
| CLAIMS | NUMBER FILED | NUMBER EXTRA | RATE | $ | |
| Total claims | 12 - 20 = | | x $ 18.00 | $ 0.00 | |
| Independent claims | 02 - 03 = | | x $ 84.00 | $ 0.00 | |
| MULTIPLE DEPENDENT CLAIM(S) (if applicable) | | | +$280.00 | $ 280.00 | |
| | | | **TOTAL OF ABOVE CALCULATIONS =** | $1,300.00 | |
| ☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2. | | | | $ 0.00 | |
| | | | **SUBTOTAL =** | $1,300.00 | |
| ☐ Processing fee of $130 for furnishing the English translation later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). | | | | $ 130.00 | |
| | | | **TOTAL NATIONAL FEE =** | $1,430.00 | |
| Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property | | | | $ | |
| | | | **TOTAL FEES ENCLOSED =** | $1,430.00 | |
| | | | | Amount to be refunded: | $ |
| | | | | charged: | $ |

a. ☒ A check in the amount of $ 1,430.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-3690. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b) must be filed and granted to restore the application to pending status.**

SEND ALL CORRESPONDENCE TO:

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Alexandria, Virginia 22320-0290

Date: March 28, 2002

Mary J Breiner
SIGNATURE

Mary J. Breiner
NAME

33,161
REGISTRATION NUMBER

# Device and Method for Characterizing Spheroids

The present invention relates to a device and a method for characterizing cell structures aggregated under microgravitation conditions. Under microgravitation conditions aggregated 3D cell structures, so-called spheroids, can be employed as models for manners of proceeding in gene technology and pharmacology.

Using spheroids as models for manners of proceeding in pharmacology and gene technology requires characterizing them with regard to the effect of drugs respectively of gene manipulations.

Presently molecular-biological methods, such as for example nucleinic acid hybridization or utilization of antibodies are employed for characterizing. Evaluation can occur by means of fluorescence microscopy. For this, however, complicated slides have to be prepared.

This method of characterizing spheroids is therefore complicated and requires experienced skilled staff for evaluation. A high throughput, desirable particularly in industrial application, automation and nondestructive characterizing are not possible with the prior art methods.

The object of the present invention is to provide a device and a method for characterizing spheroids, which permits a high throughput, automation as well as nondestructive characterization of spheroids.

The object is solved using the device and the method according to claim 1 respectively claim 7. Advantageous embodiments of the method and the device are the subject matter of the subclaims.

The invented method and the invented device is based on characterizing spheroids by using impedance spectroscopy.

Hitherto bio-impedance measuring was employed to characterize and monitor tissue damage and organ damage, for skin studies as well as tumor research and dental research. For example, electrodes were brought directly into contact with the tissue. Impedance spectrograms of cultivated cell structures were made, in that the cell structures were cultivated on planar electrode substrates and the impedance between the electrodes was determined or in that the cell cultures were cultivated on filter membranes and the impedance

was determined through the cell layer and the filter membrane (cf. e.g. J. Wegener et al., J. Biochem. Methods 32 (1996), 151-170). Proceeding in this manner is not possible with spheroids.

In the invented method, the spheroids are introduced into a tube having a smaller inner diameter than the diameter of the to-be-characterized spheroids in at least one region of their longitudinal axis, referred to hereinafter as positioning region. In this positioning region, the tube is composed either completely of an electrically insulating material or is provided with electrically insulating properties on its inner circumference, for example due to a coating with an insulating layer.

The tube, for example a capillary, is first filled with a culture medium free of any air bubbles. Then the spheroid is introduced into the narrow positioning region of the tube in such a manner that, due to the smaller inner diameter of the tube, it is in mechanical contact with the inner walls of the tube over the entire circumference. Then a current flow is generated along the tube axis over the culture medium and the spheroid by the introduced electrodes and the voltage drop over the spheroids is measured. The impedance is formed by the current and the voltage. In order to produce an impedance spectrogram, the impedance of the spheroid is usually determined over a coherent frequency region.

A relationship that can be utilized for characterization can be produced between the impedance spectrogram and the build up of spheroids respectively its change, for example, in the region of the cell membrane, the cytoplasm or the intracellular space,

In the invented method, impedance spectroscopy of spheroids is permitted, in particular, by the spheroid having mechanical contact over the entire circumference with the electrically insulating inner wall of the tube so that no current can flow past the spheroid over the culture medium or other paths when feeding in the current, which would lead to faulty measuring resultss. Due to this arrangement, the current always flows through the spheroid. Thus, impedances and impedance spectra of spheroids can be measured with high sensitivity. In this manner, the rapid and nondestructive characterizing of these spheroids is possible. In particular, parameters for automatic test systems can also be gained from the impedance spectra so that testing the effect of drugs and genetic manipulations can be realized on spheroids with a high throughput.

The invented arrangement consists of the tube is composed of an electrically insulating material or coated with a corresponding coating - at least in the positioning region - and has in the positioning region, where the spheroid is positioned during measuring, an inner diameter that is smaller than the diameter of the to-be-characterized spheroid. A first pair of electrodes having an inner and an outer electrode is disposed on one side of this region. Disposed on the second side of the positioning region located opposite in direction of the longitudinal axis of the tube, is a second pair of electrodes having an inner and an outer electrode. In each case, the inner electrode lies closer to the positioning region than the outer electrode. The electrodes can be placed at the inner circumference of the tube or can extend along the inner volume of the tube.

Furthermore, the device is provided with a measuring arrangement for feeding in an alternating current between the two outer electrodes and for determining the resulting alternating voltage between the two inner electrodes. Of course, all the electrodes have to be contactable from outside the tube. The measuring arrangement can, for example, consist of a commercially obtainable impedance analyzer.

The invented device permits rapid and nondestructive characterization of spheroids. Due to the arrangement with the smaller small tube diameter for positioning the spheroids and the pairs of electrodes disposed on both sides in the longitudinal direction of the tube, the shunt paths have very high resistance and, due to the arrangement of the separated electrodes, the influence of electrode polarization is negligible for generating the current flow and measuring the voltage. It is particularly due to this that the impedance of the spheroids, which usually have low resistance, can be determined with high sensitivity.

Of course, carrying out the measurement, the diameter of the tube has to be adapted to the diameter of the spheroids - or inversely, because too small spheroids would not be in contact with the inner wall of the tube over the entire circumference. The size of the spheroids lies usually in the range between 0.1 and 0.5mm so that the diameter of the tube has to lie in the same range.

Preferably a plurality of tubes of different diameters are at disposal for characterizing spheroids of different sizes. The individual spheroids can, for example, be preselected according to size by means of a perforated screen, which ensures a reproducible

measurement in which the spheroids are always pressed into the tube to the same degree.

Preferably the tube has a conical-shaped enlargement on one or both sides of the positing region permitting simple and rapid introduction of the spheroids into the positioning region without any damage. The electrodes are preferably disposed in the conical-shaped enlarged region and extend radially into the tube. Due to this enlargement in this region, the electrodes do not hinder introduction of the spheroids.

For positioning the spheroids in the tube, the spheroids are preferably pressed into or drawn into the tube via a pump acting on the culture medium. Control of the correct position can occur by optical means.

In a preferred embodiment, a current flow, however, is generated by the electrodes during the positioning procedure and the resulting resistance is measured. If the spheroids are positioned correctly, this resistance increases markedly. This control can, for example, occur by means of measuring the direct current resistance.

Of course, the tube can also be designed conical-shaped in the positioning region so that it is possible to characterize spheroids having different diameters which attach themselves at different points of the conical-shaped positioning region. However, the reproducibility problem arises here, because the degree of compression of the spheroids and, thus, their length respectively their resistance along the tube axis depends on the pressure force. This problem can be avoided with a constant tube diameter.

In another preferred embodiment, a tube is provided in which the inner diameter changes in the positioning region in steps along the longitudinal axis. Spheroids of different sizes can also be attached by this means.

The invented device and the corresponding method permit measuring a spheroid in a very short time. Measurement of the impedance can be conducted in less than 1 second. Positioning time lies in the range of a few minutes or less.

Especially for industrial use, an array-like arrangement of a multiplicity of invented devices is advantageous, when, for example, they have different diameters. A multiplicity of spheroids can be

characterized in parallel by this means. Furthermore, the use of tubes with a constant cross section over the positioning region permits introduction of the spheroids from one side of the tube and expulsion of the spheroids after measuring on the opposite side of the tube so that continuous throughput can be achieved in automatic measuring systems.

A preferred field of application of the present method respectively of the corresponding device is in the field of (chemo) therapeutic testing (pharmacology, pharmakinetics; side effects) and their effect mechanisms. For example, proof of gene-therapeutic approaches on cancer tumor spheroids can be conducted with it. With the aid of impedance spectroscopy using the present method respectively device with a positioned gene-manipulated tumor cell spheroid permits determining morphological changes, disintegration of the tissue and an increase in necrotic areas from the impedance changes in the cell membrane in the shortest time in a reproducible manner. Thus, use of the present device offers a rapid and efficient method of proving the effectiveness of gene constructs for use in tumor gene therapy.

The present invention is described once more in the following using preferred embodiments with reference to the accompanying drawings, showing in:

Fig. 1: a cross section of a detail of a preferred embodiment of an invented device with a positioned spheroid;

Fig. 2: a diagrammatic representation of a preferred embodiment of the invented device for characterizing spheroids by means of impedance spectroscopy; and

Fig. 3a/b: . a cross section of two further examples of the geometric shape of the tube of the invented device.

In this preferred embodiment, the invented device consists of a tube having an inner diameter of 0.2mm in the positioning region of the spheroid and an inner diameter of 4mm outside this positioning region. Such a type of tube, as shown in figure 1, can be produced from a narrow capillary 1 of insulated material, as for e.g. glass to both ends of which, glass tubes 2 having a larger diameter are fused.

In this example, the capillaries have a length of 8mm and the small glass tubes a length of 40mm. The

transition of the inner diameter of the small glass tubes 2 and the glass capillaries runs conical-shaped.

In the two fused-on small glass tubes 2, on both sides of the positioing regions, a first borehole is provided at a distance of 15 mm from the center of this region and a second borehole at a distance of 20m from the center of this region respectively. The boreholes have a diameter of 0.4mm. Four platinum wires 3,4 with a length of 10cm and a diameter of 0.3mm are glued into the boreholes.

The platinum wires form the outer electrodes 4 respectively the inner electrodes 3 for receiving the impedance spectogram. The given distances of the electrodes from the entrance of the tube are, of course, only intended as an example and have no significant influence on the measurement. The electrodes may also be disposed in the tube in another manner, for example, as a coating.

Moreover, figure 1 shows the culture medium 5 filled into the tube without any air bubbles and the positioned spheroid 6 pressed into the positioning region. For conducting the measurement, an alternating current is applied to the two external electrodes 4. The drop in alternating voltage over the spheroid is detected by the two inner electrodes 3 .

Figure 2 depicts a diagram of an example of the entire invented device. This figure shows the narrow positioning region of the glass capillary 1, the two outer glass tubes 2 having a large inner diameter and the outer electrodes 4 and inner electrodes 3. In order to introduce the spheroid, the glass body 1,2 is attached with the electrodes at a holding means. The lower opening of the glass tube is connected by means of a flexible tube 7 filled with the culture medium 5, in this case having a length of 20cm and an inner diameter of 5mm, to a fine-control valve 8 having a pressure-release valve.

The culture medium is pressed from the flexible tube into the glass body 1,2 via the fine-control valve until the glass body is completely filled with the culture medium 5. Then the to-be-characterized spheroid 6 is introduced into the culture medium through the upper opening of the glass body. After this a flexible tube 9, which is filled with oil, is connected to the upper opening of the glass body. The flexible tube filled with oil is connected with its other end to a moveable piston 10. Then the pressure-release valve 8 is opened. If the spheroid 6 has sunk due to the gravitational force in the conical-shaped

transition from the glass tube 2 to the capillary 1, the spheroid is centrally positioned by suited operation of the moveable piston 10. The central position of the spheroid is shown in the figure. In order to position the spheroid, differences in pressure are generated in the capillary by means of the moveable piston via the oil-filled flexible tube. After positioning, the spheroid 6 remains in the corresponding position into which it was pressed.

The four platinum electrodes 3, 4 are connected to an impedance analyzer, consisting of a current source 11 and a voltage meter 12. Via the outer electrode 4s, a current $i = I^* \times \sin(\omega t + \varphi_i)$ is fed in, which is regulated in such a manner that the drop in voltage $u = U^* \times \sin(\omega t + \varphi_u)$ over the inner electrodes 3 is approximately 10 mV. This alternating voltage is detected by means of the voltage meter 12. The impedance analyzer derives the amount and the phase of the impedance from the current and the voltage. In order to obtain the characteristic impedance spectrum of the spheroid 6, the impedances are determined over the frequency range of 20 Hz to 1 Mhz.

Figures 3a and 3b show two further examples of the geometric shape of the tube of the invented device. The tube cross section changes steplike over the positioning region 1. In addition, the embodiment of figure 3b is provided with bulges which prevent the spheroid 6 from changing position if , for example, light forces unintentionally act on the spheroid via the liquid surrounding the spheroid.

Both the embodiments permit taking up spheroids 6 of various sizes, as the figures distinctly indicate. Of course, always only one spheroid is placed in the tube during measuring. The three spheroids 6 shown in the figures are depicted simultaneously only for illustration. Preselection of the spheroids according to size is not necessary if the tube has this shape. Control of the correct positioning can occur, for example optically or electrically as previously explained herein.

What Is Claimed Is:

1. A device for characterizing spheroids comprising
- a tube (1, 2) which has an inner diameter in a region (1) of its longitudinal axis which is smaller than the diameter of the to-be-characterized spheroid (6), with said tube being composed of an electrically insulating material at least at its inner circumference;
- a first pair of electrodes (3, 4) in said tube (2) on one first side of said region (1) and a second pair of electrodes (3, 4) in said tube (2) on a second side of said region (1), which lies opposite said first side, with each pair of electrodes (3,4) having an inner electrode (3) and an outer electrode (4) of which said inner electrode (3) lies closer to said region (1) than said outer electrode (4); and
- a measurement arrangement (11,12) having a current source (11) which is connected to the outer electrodes (4) and a voltage meter (12) which is connected to the inner electrodes (3).

2. A device according to claim 1, wherein said tube (1,2) has a conical-shaped enlargement on one side or on both sides of said region (1).

3. A device according to claim 1 or 2, wherein said electrodes (3,4) extend radially into said tube (2).

4. A device according to one of the claims 1 to 3, wherein in said region (1), said tube (1,2) has an inner diameter of between 0.1 and 0.5mm .

5. A device according to one of the claims 1 to 4, wherein said tube (1,2) is composed of glass.

6. A device according to one of the claims 1 to 5, wherein said inner diameter of said tube (1,2) changes step-like in said region (1).

7. A method for characterizing spheroids having the following steps: - provision of a tube (1,2) which has in region (1) an inner diameter which is smaller than the diameter of the to-be-characterized spheroid, with said tube in said region (1) being composed of an electrically insulating material at least at its inner circumference;
- filling said tube (1,2) with a liquid culture medium (5);
- introduction of a spheroid (6) into said region (1)

of said tube (1,2) in such a manner that the latter has mechanical contact over the entire circumference with the inner wall of said region (1) of said tube (1,2);
- generation of an alternating current flow in said culture medium (5) along the longitudinal axis of said tube over said spheroid (6); and
- measurement of the drop in alternating voltage over said spheroid (6) along said longitudinal axis of said tube.

8. A method according to claim 7, wherein the introduction of said spheroid (6) is conducted by drawing in or pressing in by means of generating a difference in pressure in said culture medium (5).

9. A method according to one of the claims 7 or 8, wherein correct positioning of said spheroid (6) in said region (1) occurs by means of generating and measuring a direct current along said longitudinal axis of said tube in said region (1) during introduction of said spheroid (6).

10. A method according to one of the claims 6 to 9, wherein said to-be-characterized spheroid (6) is preselected according to size by means of a perforated screen.

## Abstract

The present invention relates to a device and a method for characterizing spheroids.

The to-be-characterized spheroid (6) is introduced into a tube (1, 2) of an electrically insulating material and with an inner diameter which is smaller than the diamter of the to-be-characterized spheroid (6) in such a manner that the latter is in mechanical contact over the entire circumference with the inner wall of the electrically insulated tube (1,2). Electrodes (3, 4) are disposed on both sides of the spheroid (6) in the tube. By means of these electrodes, a current flow can be generated through the spheroid (6) and the resulting drop in voltage at the spheroid can be measured. The invented arrangement and the corresponding method permits producing an impedance spectrum of spheroids with high sensitivity, thereby realizing rapid and nondestructive characterization of spheroids. (figure 2).

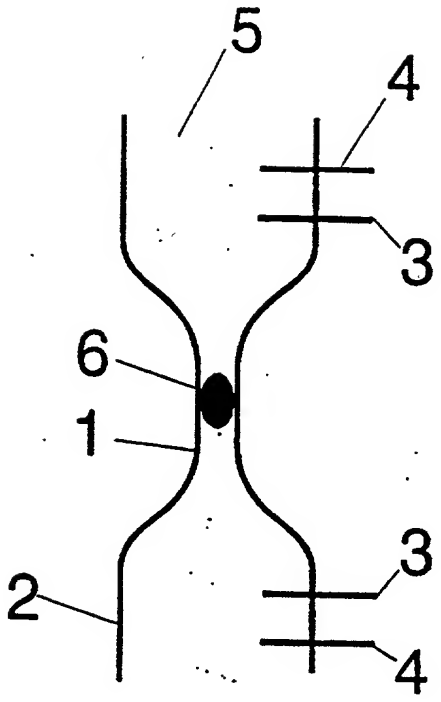
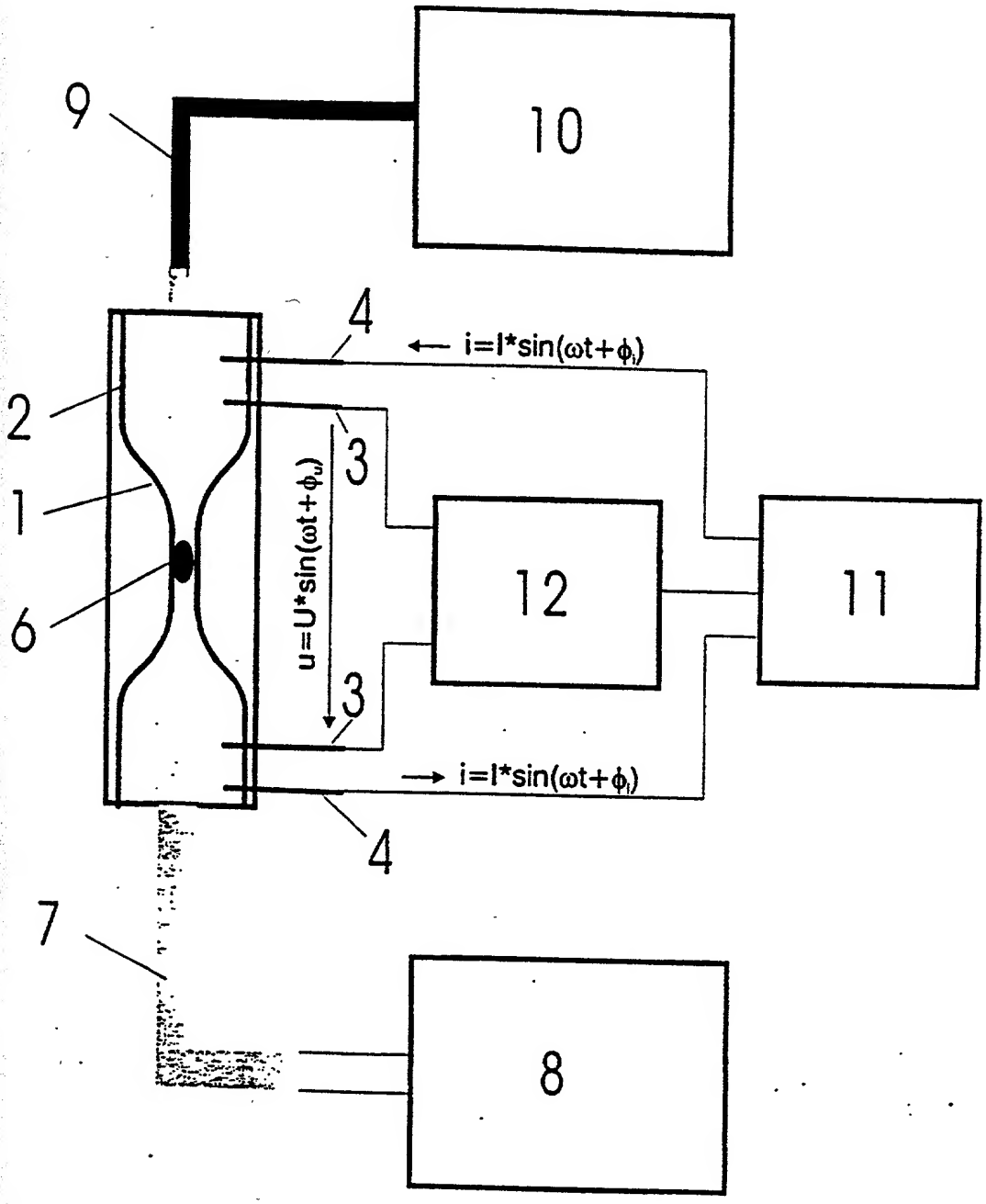



Figure 1


9
10
4
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2
3
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12
11
6
3
$i=I*\sin(\omega t+\phi_i)$
4
7
8

Figure 2

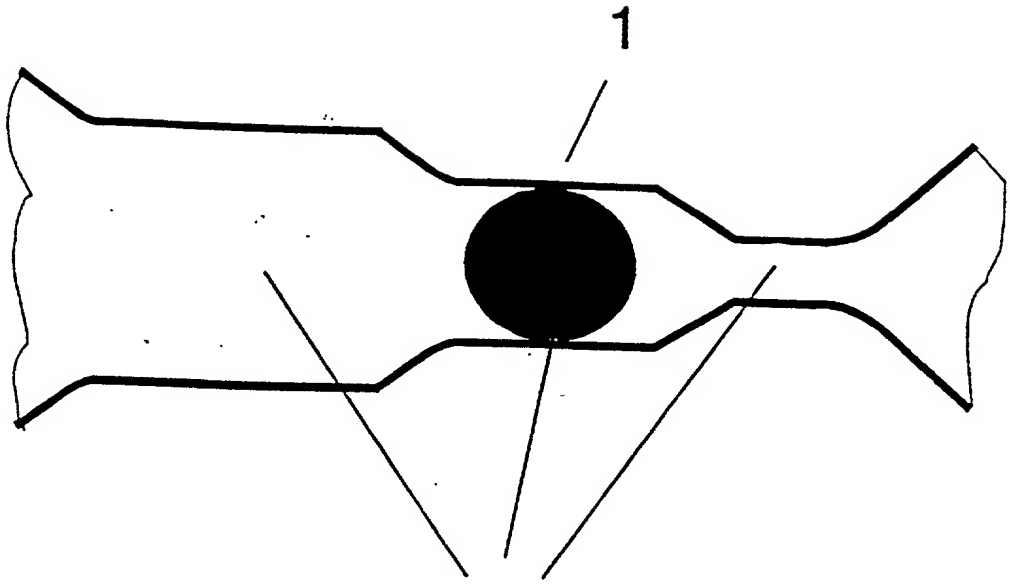


Figure 3a

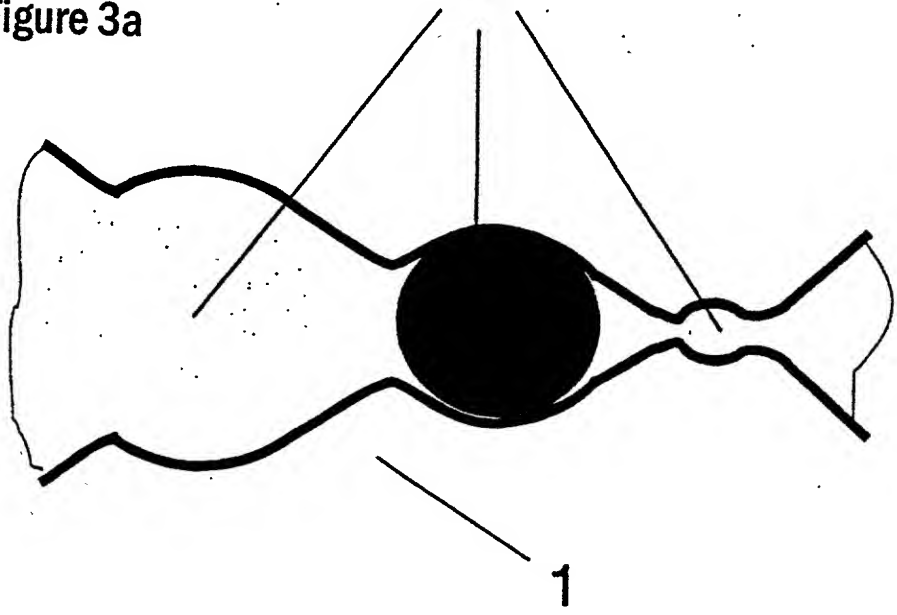


Figure 3b

COMBINED DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION

Docket No. 5686

As a below named inventor, I hereby declare that:

My residence, Mailing Address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled DEVICE AND METHOD FOR CHARACTERIZING SPHEROIDS

_____,

the specification of which is attached hereto unless the following box is checked:

[X] was filed on August 12, 2000 as United States Application Number or PCT International Application Number PCT/DE00/02753 and was amended on July 19, 2001 (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

| Prior Foreign Application(s) | | | Priority Claimed |
|---|---|---|---|
| 199 46 458.8 | Germany | 28 September 1999 | Yes [X] No [ ] |
| (Number) | (Country) | (Day/Month/Year Filed) | |
| PCT/DE00/02753 | PCT | 12 August 2000 | Yes [X] No [ ] |
| (Number) | (Country) | (Day/Month/Year Filed) | |
| _____ | _____ | _____ | Yes [ ] No [ ] |
| (Number) | (Country) | (Day/Month/Year Filed) | |

COMBINED DECLARATION & POWER OF ATTORNEY Docket No. 5686

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

| (Application Number) | (Filing Date) |
|---|---|
| (Application Number) | (Filing Date) |

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s), or §365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

| (Application No.) | (Filing Date) | (Status-patented, pending, abandoned) |
|---|---|---|
| (Application No.) | (Filing Date) | (Status-patented, pending, abandoned) |

I (we) hereby appoint the following attorney with full power of substitution to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

3 ALFRED W. BREINER, Reg. No. 18,676; THEODORE A. BREINER, Reg. No. 32,103; MARY J. BREINER, Reg. No. 33,161; and C. BRANDON BROWNING, Reg. No. 44,570.

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P.O. Box 19290, Alexandria, Virginia 22320-0290

Address all telephone calls to -

Mary J. Breiner at (703) 684-6885

COMBINED DECLARATION & POWER OF ATTORNEY Docket No. 5686

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Full Name of Sole or First Inventor:
(given name, family name) Hagen THIELECKE

Inventor's Signature Hagen Thielecke Date 6 May 2002

Residence: St. Ingbert, GERMANY DEX Citizenship: Germany

Mailing Address: Hasseler Chaussee 23, D-66386 St. Ingbert, GERMANY

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Full Name of Second Joint Inventor, if any
(given name, family name) Andrea ROBITZKI

Inventor's Signature Andrea Robitzki Date 6 May 2002

Residence: Viernheim, GERMANY DEX Citizenship: Germany

Mailing Address: Brunhildstr. 8, D-68519 Viernheim, GERMANY

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Full Name of Third Joint Inventor, if any
(given name, family name)

Inventor's Signature Date

Residence: Citizenship:

Mailing Address:

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